Epidemiol. Infect. (1994), **112**, 45–49

Bacteriophage typing in *Salmonella bareilly*

N. C. SHARMA[1], R. BHATIA[2], S. SINGH[1], P. C. JOHN[1], S. KUMAR[1] AND H. SINGH[1]*

[1] *National Salmonella and Escherichia Centre, Central Research Institute, Kasauli-173 204, India*

[2] *Department of Microbiology, Safdarjang Hospital, New Delhi, India*

(*Accepted 3 September 1993*)

SUMMARY

A total of 675 strains of *Salmonella bareilly* received from different parts of India and France during 1959–92 were phage typed using six bacteriophages. Overall typability achieved was 90·8% with 23 distinct phage types excluding a group of untypable strains. Phage types have been defined in octal code. Simpson's coefficient was applied for diversity index having a value of 0·839. This system was found to be reproducible, stable and epidemiologically useful.

INTRODUCTION

In spite of the availability of newer methods to fingerprint *Salmonella* serotypes [1] phage typing is still considered a valuable epidemiological tool [2–4]. *Salmonella bareilly* was isolated for the first time in India in 1928 [5] and remained a rarely isolated serotype until 1980 [6, 7] with a solitary outbreak reported to that time [8]. Since 1980, *S. bareilly* has been frequently isolated from human as well as non-human sources [8–18] and this trend is still maintained [19]. *S. bareilly* infections have been recorded in more than 35 countries [20] and this has necessitated development of a system of epidemiological tracing. Early attempts to develop phage-typing systems for *S. bareilly* did not yield desirable results (21–23]. With the availability of six phages isolated in our centre [23, 24] we have developed a possible phage-typing scheme for *S. bareilly*.

METHODS

Bacteriophages

One sewage sample yielded 12 prospective phage preparations of which Sab W1 and Sab W2 were found useful. Five strains of *S. bareilly* were examined for lysogenic phages and eight phages were isolated. Only three of these, Sab L1, Sab L2 and Sab L3, were found suitable for typing purposes [24]. Phages were purified by seven single-line plaque isolation using the soft overlay agar method [25], and routine test dilutions (RTD) were determined by 10-fold serial dilutions [26]. Bacteriophage Sab 2 obtained from an earlier study [23] was also included in the battery of the above phages.

Phage-typing

Phage-typing was carried out by spot test [27] using phage preparations at their RTDs and results were recorded as described elsewhere [24].

Bacterial strains

Six hundred and twenty-five strains of *S. bareilly* isolated from human, animal and other sources in India during 1959–92 and 50 strains obtained from Dr P. A. D. Grimont of the National Salmonella Centre, Paris, France, were phage typed.

Media

The phage broth, phage agar, soft agar and diluent used have been previously reported [22].

Reproducibility and stability

Reproducibility and stability of the results were checked by repeating phage-typing of all the strains of *S. bareilly* after storage at 22 °C for 6 months.

Octal code

Octal weight was assigned to each phage, and phage types were defined in terms of octal code for reporting in the present scheme [28].

Diversity index

The discrimination power was calculated by Simpson's index of diversity [29] according to the formula [32]

$$D = 1 - \sum (Ni[Ni-1])/N(N-1).$$

RESULTS

Phage Sab W2 lysed 85·2% of the strains with further subdivision produced using Sab W1, Sab L3, Sab 2, Sab L1 and Sab L2 phages. This set of phages gave 90·8% typability excluding a group of untypable strains. The prevalence of phage types per octal code are shown in Table 1. The majority of strains have been grouped into phage types 10, 73, 71, 14 and 12.

Reproducibility and stability were evaluated by repeating the phage-typing experiments on stored cultures. Only 10 strains had changes in their phage-typing pattern. The diversity index was 0·839.

DISCUSSION

Three previous attempts have been made to develop a phage-typing scheme for *S. bareilly* [21–23]. In the first attempt [21] an insufficient number of strains was used to develop a scheme and the second attempt based on lysogenotyping [22] was indirect, laborious and its typability was limited to 70·3%. Subsequently a scheme was developed using wild phages [23] but this had the disadvantage of poor discrimination [30]. None of the above schemes indicated the level of their reproducibility and stability which are important for any typing system [31, 32]. In the present scheme reproducibility and stability could be demonstrated with minor variation (1·48%) which has been well documented [30]. The discrimination

Table 1. *Phage-typing scheme for* Salmonella bareilly

Bacteriophages*						Phage type in octal code	Number of strains	%
Sab W2	Sab W1	Sab L3	Sab 2	Sab L1	Sab L2			
1†	2	4	1	2	4			
+	+	+	+	+	+	77	6	0·9
+	+	+	+	−	+	75	3	0·4
+	+	+	−	−	+	74	12	1·7
+	+	+	+	+	−	73	108	16·0
+	+	+	+	−	−	71	88	13·0
−	+	+	+	+	+	67	2	0·3
−	+	+	+	−	+	65	2	0·3
−	+	+	−	−	+	64	1	0·15
−	+	+	+	+	−	63	3	0·4
−	+	+	+	−	−	61	7	1·0
−	+	+	−	−	−	60	2	0·3
+	−	+	−	−	−	50	2	0·3
+	+	−	−	−	+	34	5	0·7
+	+	−	−	+	−	32	15	2·2
+	+	−	+	−	−	31	1	0·15
+	+	−	−	−	−	30	13	1·9
−	+	−	−	−	−	20	12	1·7
+	−	−	−	−	+	14	69	10·2
+	−	−	−	+	−	12	48	7·1
+	−	−	−	−	−	10	206	30·5
−	−	−	+	−	+	05	1	0·15
−	−	−	−	−	+	04	4	0·6
−	−	−	−	+	−	02	3	0·4
−	−	−	−	−	−	00	62	9·1
Total 576	280	236	221	185	105		675	
% 85·2	41·4	34·9	32·7	27·3	15·5			

*−, No plaques or < 20 plaques; + > 20 plaques to confluent lysis.
† Octal weight.

index was evaluated (0·839) with an 83·9% chance of any two randomly sampled strains from the population falling into different types. An index of 0·90 is considered desirable in typing systems [32]. The majority of the strains (76·8%) were grouped into five different phage types (10, 73, 71, 14 and 12) by the current scheme, as compared to 84% of the strains grouped into two phage types in an earlier scheme [23, 30]. Phages used in the present system had been extensively studied for their host range and were characterized prior to development of the scheme, and were found to be highly specific for *S. bareilly* strains [24]. Moreover, lysogenic phages are more specific as compared to wild phages [33] and the present typing set consists of both wild as well as lysogenic phages. The scheme described here shows definite improvement over the past schemes developed for this serotype.

Epidemiological evaluation of the scheme revealed that 21 strains isolated from different animals (tortoise, lizard, toad, fish) received on two different occasions from Mohanpur, Dist Nadia (West Bengal) fell into phage type 73. It was also revealed from the data that in 1983 phage type 71 was prevalent in Delhi. This was replaced by phage type 10 in the year 1984 which remained the predominant type

in 1985 and 1986. Subsequently in 1987 phage types 14 and 20 appeared including strains belonging to an untypable group (00). Untypable strains were also found to be epidemiologically related. Strains belonging to group 00 and type 10 remained prevalent till 1990 and in 1992 only phage type 20 was encountered.

Fifteen strains received from Goa on two occasions were grouped into one phage type (14). In all, the epidemiological usefulness of the scheme was proved on at least 55 occasions, but it was not possible to give a full account of all these. Phage preparations used in the present scheme are being maintained for further evaluation in this Institute.

REFERENCES

1. Threlfall EJ, Frost JA. The identification, typing and fingerprinting of *Salmonella*: laboratory aspects and epidemiological applications. J Appl Bacteriol 1990; **68**: 5–16.
2. de Sa JDH, Ward LR, Rowe B. A scheme for the phage typing of *Salmonella hadar*. FEMS Microbiol Lett 1980; **9**: 175–7.
3. Chambers RM, McAdam P, de Sa JDH, Ward LR, Rowe B. A phage typing scheme for *Salmonella virchow*. FEMS Microbiol Lett 1987; **40**: 155–7.
4. Ward LR, de Sa JDH, Rowe B. A phage typing scheme for *Salmonella enteritidis*. Epidemiol Infect 1987; **99**: 291–4.
5. Bridges RF, Scott WM. A new organism causing paratyphoid fever in India, *Salmonella* type 'Bareilly'. J R Army Med Corps 1931; **56**: 241–9.
6. Basu S, Dewan ML, Suri JC. Prevalence of *Salmonella* serotypes in India – 16 years study. Bull WHO 1975; **52**: 331–6.
7. Saxena SN, Ahuja S, Mago ML, Singh H. Salmonella pattern in India. Indian J Med Res 1980; **72**: 159–68.
8. Sharma VK, Singh CM. *Salmonella bareilly* and *S. chester* from an outbreak of disease in chicken. Indian J Microbiol 1963; **3**: 85–6.
9. Agarwal KC, Garg RK, Panhotra BR, Verma AD, Ayyagari A, Mahanta J. Drug resistance in *Salmonella* isolated at Chandigarh (India) during 1972–1978. Antonie van Leeuwenhoek 1980; **46**: 383–90.
10. Kapoor KN, Chauhan HVS, Gupta BR. Epidemiological and pathological studies in outbreaks of *Salmonella bareilly* infection in chickens and quails. Indian Vet J 1980; **57**: 536–8.
11. Paul SS, Pawa RR, Verma M, Singh D. Outbreak of *Salmonella* infection in paediatric department. Indian Pediatr 1981; **10**: 899–904.
12. Panhotra BR, Agarwal KC. Urinary tract infection caused by *Salmonella typhimurium* and *Salmonella bareilly*. Indian J Med Res 1982; **76**: 62–4.
13. Raichowdhuri AN, Aggarwal P, Singh M, Anand BR. Salmonella aetiology of acute diarrhoea. J Commun Dis 1983; **15**: 8–13.
14. Aggarwal P, Sarkar R, Singh M, Grover BD, Anand BR, Raichoudhuri AN. *Salmonella bareilly* infection in a paediatric hospital of New Delhi. Indian J Med Res 1983; **78**: 22–5.
15. Ram S, Khurana S, Vadehra DV, Sharma S. Bioecological factors and *Salmonella* diarrhoea. Indian J Med Res 1987; **86**: 441–50.
16. Shah PR, Patel HH. Bacteria associated with sporadic cases of acute diarrhoea in children of rural Panchmahals. Indian J Microbiol 1988; **28**: 116–22.
17. Saxena SN, Jayasheela M, John PC, Mago ML, Kumari Neelam, Sharma NC. *Salmonella* serotypes in India, 1982–1983. Indian J Pathol Microbiol 1988; **31**: 286–97.
18. Saxena SN, Jayasheela M, John PC, Mago ML, Sharma, NC. *Salmonella* serotypes in India, 1984–1985. Indian J Pathol Microbiol 1990; **33** (suppl): 67–76.
19. Saxena SN, Jayasheela M, John PC, Soni, NK. *Salmonella* serotypes in India 1986–1989. Indian J Med Microbiol 1991; **9**: 118–30.
20. Kelterborn E. *Salmonella* species. The Hague: Dr W Junk, 1967: 67.
21. Majumdar AK, Singh SP. A phage-typing scheme for *Salmonella bareilly*. Indian Vet J 1973; **50**: 1161–6.
22. Sharma NC, John PC, Mago ML, Saxena SN. Phage-typing scheme of *Salmonella bareilly* based on lysogeny. Antonie van Leeuwenhoek 1984; **50**: 275–9.

23. Jayasheela M, Singh G, Sharma NC, Saxena SN. A new scheme for phage typing *Salmonella bareilly* and characterisation of typing bacteriophages. J Appl Bacteriol 1987; **62**: 429–32.
24. Sharma NC. Intraserotypic differentiation in *Salmonella bareilly* by using different epidemiological markers. [dissertation]. Shimla, India: Himachal Pradesh University, 1989.
25. Adams MH. Bacteriophages. New York: Interscience Publishers, 1966: 505–8.
26. Anderson ES, Williams REO. Bacteriophage typing of enteric pathogens and Staphylococci and its use in epidemiology. J Clin Pathol 1956; **9**: 94–127.
27. Boyd JSK. The symboitic bacteriophages of *Salmonella typhimurium*. J Pathol Bacteriol 1950; **62**: 501–17.
28. Audurier A, Chalons F, Toucas M. A code for reporting and comparing results in phage typing. Ann Microbiol (Inst Pasteur) 1979; **130A**: 345–9.
29. Simpson EH. Measurement of diversity. Nature 1949; **163**: 688.
30. Singh G, Sharma NC, Jayasheela M, Saxena SN. A study of the epidemiology of *Salmonella bareilly* in India using a new phage-typing system. Epidemiol Infect 1988; **100**: 221–5.
31. Guinee PAM, van Leeuwen WJ. Phage typing of *Salmonella* In: Bergen T, Norris JR, eds. Methods in microbiology vol 11. New York: Academic Press, 1978: 157–91.
32. Hunter PR, Gaston M. Numerical index of the discriminatory ability of typing schemes: an application of Simpson's index of diversity. J Clin Microbiol 1988; **26**: 2465–6.
33. Ackermann H.-W, Du Bow MS. Viruses of prokaryotes, vol 1 Boca Raton: CRC Press, 1987: 143–72.